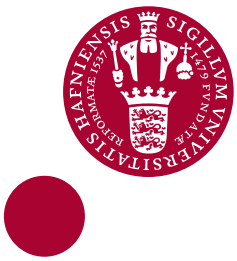


Evaluating the microbial effects of stocking freshwater snails (Physa gyrina) in water reuse systems culturing rainbow trout (Oncorhynchus mykiss)

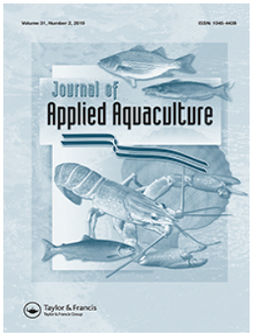
Davidson, John; Plautz, Carol Zygar; Grimm, Casey; Jørgensen, Niels O. G.; Podduturi, Raju; Raines, Clayton; Snader, Ryan; Summerfelt, Steven; Good, Christopher

Published in:
Journal of Applied Aquaculture

DOI:
10.1080/10454438.2018.1541771

Publication date:
2019

Document version
Publisher's PDF, also known as Version of record



Journal of Applied Aquaculture

ISSN: 1045-4438 (Print) 1545-0805 (Online) Journal homepage: https://www.tandfonline.com/loi/wjaa20

Evaluating the microbial effects of stocking freshwater snails (*Physa gyrina*) in water reuse systems culturing rainbow trout (*Oncorhynchus mykiss*)

John Davidson, Carol Zygar Plautz, Casey Grimm, Niels O.G. Jørgensen, Raju Podduturi, Clayton Raines, Ryan Snader, Steven Summerfelt & Christopher Good

To cite this article: John Davidson, Carol Zygar Plautz, Casey Grimm, Niels O.G. Jørgensen, Raju Podduturi, Clayton Raines, Ryan Snader, Steven Summerfelt & Christopher Good (2019) Evaluating the microbial effects of stocking freshwater snails (*Physa gyrina*) in water reuse systems culturing rainbow trout (*Oncorhynchus mykiss*), Journal of Applied Aquaculture, 31:2, 97-120, DOI: 10.1080/10454438.2018.1541771

To link to this article: https://doi.org/10.1080/10454438.2018.1541771

JOURNAL OF APPLIED AQUACULTURE
2019, VOL. 31, NO. 2, 97–120
https://doi.org/10.1080/10454438.2018.1541771

Taylor & Francis
Taylor & Francis Group

Evaluating the microbial effects of stocking freshwater snails (*Physa gyrina*) in water reuse systems culturing rainbow trout (*Oncorhynchus mykiss*)

John Davidson [a], Carol Zygar Plautz [b], Casey Grimm [c], Niels O.G. Jørgensen [d], Raju Podduturi [d], Clayton Raines [a], Ryan Snader [a], Steven Summerfelt [a], and Christopher Good [a]

[a]The Conservation Fund Freshwater Institute, Shepherdstown, West Virginia, USA; [b]Department of Biology, Shepherd University, Shepherdstown, West Virginia, USA; [c]Food Processing and Sensory Quality Research Unit, USDA Agricultural Research Service, New Orleans, Louisiana, USA; [d]Department of Plant and Environmental Science, University of Copenhagen, Copenhagen, Denmark

ABSTRACT

A study was conducted to analyze the effects of snails (*Physa gyrina*) on biofilm, bacterial abundance, off-flavor-producing bacteria, and off-flavor compounds in reuse aquaculture systems culturing rainbow trout (*Oncorhynchus mykiss*). Eight experimental-scale systems were used, including four with and without snails. Mean heterotrophic bacteria counts in water were lower ($P < 0.05$) in systems with snails. Submerged surfaces of sumps containing snails were nearly biofilm-free, while sumps without snails were coated with biofilm. Geosmin levels in trout fillets from snail-stocked systems were generally lower but not statistically different from the controls. Rainbow trout health and performance was not affected by snail presence.

KEYWORDS

Biofilm; snails; off-flavor; geosmin; water reuse; recirculating aquaculture systems

Introduction

Biofilm is as an assemblage of bacterial colonies contained within a protective extracellular matrix that adheres to a surface. Biofilm grows on a variety of surfaces and substrates and is commonly found within aquatic environments, including fish production systems. In certain types of aquaculture, biofilm can be advantageous, particularly in the production of grazing food-fish species that utilize biofilm as a food source, such as Nile tilapia (*Oreochromis niloticus*) (Shrestha and Knud-Hansen 1994), common carp (*Cyprinus carpio*) (Ramesh et al. 1999), and various shrimp species (Thompson, Abreu, and Wasielesky 2002). Biofilm formation on select media substrates is also critical for optimal nitrification in

CONTACT John Davidson jdavidson@conservationfund.org The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443, USA.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/wjaa.

Present address of Clayton Raines is West Virginia Division of Natural Resources, 1 Depot Street, Romney, WV 26757, USA
Present address of Ryan Snader is Maryland Department of the Environment, 416 Chinquapin Round Road, Annapolis, MD 21401, USA
Present address of Steven Summerfelt is Superior Fresh LLC, W15506 Superior Fresh Drive, Hixton, WI 54635, USA

recirculation aquaculture systems (RAS) that utilize biofilters (Chen, Ling, and Blancheton 2006; Hagopian and Riley 1998; Kaiser and Wheaton 1983; Wheaton, Hochheimer, and Kaiser 1991). Pandey, Vivekanand, and Kumar (2014) reviewed the reported advantages of biofilm in aquaculture production, including supplemental nutrition and increased performance of cultured species (Thompson, Abreu, and Wasielesky 2002), pathogen inhibition (Aly et al. 2008), nutritional quality and use as a feed ingredient (Kuhn et al. 2010), and vaccine development (Azad et al. 1999), among others.

While biofilm has many known advantages in aquaculture systems, a range of disadvantages has also been reported. Biofilms that form on the submerged surfaces of fish culture systems can contain harmful microorganisms, including fish and human pathogens (King et al. 2004, 2006, 2008). King et al. (2004) identified the human pathogens *Bacillus cereus, Shigella* spp., and *Vibrio* spp. and major fish pathogens *Photobacterium damsela* and *Aeromonas hydrophilla* in the biofilms of fresh and saltwater RAS. In addition, King et al. (2006) found that *Escherichia coli* bacteria became incorporated in biofilm of a RAS after artificial introduction and concluded that there is strong potential for pathogen colonization in biofilms. Cai, Fuente, and Arias (2013) also described biofilm formation by the fish pathogen *Flavobacterium columnare*, the causative agent of columnaris disease in freshwater fish under both static and flowing water conditions and indicated that virulent colonies were maintained within the biofilm.

Other studies have found that off-flavor-producing bacteria are metabolically active within submerged organic matter, particularly in aerobic zones of the water recycle loop (Guttman and van Rijn 2008; Schrader and Summerfelt 2010). Schrader and Summerfelt (2010) reported that off-flavor-producing actinomycetes were isolated from biosolids (biofilm) scrapings collected from the inside surfaces of drum filters and heat exchangers of replicated RAS. Filamentous actinobacteria, like those identified by Schrader and Summerfelt (2010), have been implicated as common producers of odorous compounds in water reuse systems (Burr et al. 2012; Guttman and van Rijn 2008; Schrader, Harries, and Page 2015) and are specifically known to produce two of the most common off-flavors, geosmin and 2-methylisoborneol (MIB), which impart earthy and musty tastes respectively to fish fillets (Howgate 2004; Petersen et al. 2011; Tucker 2000). Off-flavor problems have been reported in fish cultured in various aquaculture production technologies, including ponds (Brown and Boyd 1982), river-fed raceways (Robertson et al. 2006), partial reuse systems (Robin et al. 2006; Schrader et al. 2005; authors' experience), and RAS (Burr et al. 2012; Houle et al. 2011; Schrader, Harries, and Page 2015). This article is focused on aquaculture systems that reuse or recirculate water. No matter the culture environment, aquatic foods tainted with these off-flavors are objectionable to consumers

and can cause severe economic losses for fish farmers (Engle, Pounds, and Ploeg 1995; Hansen 2003; Tucker 2000).

Several studies have evaluated methods to mitigate off-flavor within recirculation systems used for fish growout. Guttman and van Rijn (2008) reported that off-flavor compounds were reduced within an anaerobic treatment loop of a zero-water exchange RAS, and Acuña-Rubio (2002) determined that aeration/degassing was an effective method for reduction of MIB and geosmin from RAS water. Recently, Ma et al. (2016) found that bioflocs maintained within a suspended growth reactor removed geosmin and MIB from spiked inlet flows with simulated off-flavor levels common to aquaculture water. Novel approaches have been evaluated to manage off-flavors in potable water treatment with some success, including sand filter inoculation with geosmin-degrading bacteria (McDowall et al. 2009), acetic and citric acid treatments (Pahila and Yap 2013), and advanced oxidation processes such as use of ozone, hydrogen peroxide, ultraviolet light, ultrasound, electrolysis, or combinations of these (Antonopoulou et al. 2014; Klausen and Grønborg 2010). However, adoption of potable water treatment methods by the aquaculture industry is complex because chemical dosing rates must comply with the health and welfare of aquatic organisms. Ultimately, a commercially reliable strategy for off-flavor management has yet to be developed for fish production systems that reuse water. As such, depuration systems that flush relatively high volumes of water, while fish are kept off feed, are typically required as a finishing step to purge off-flavors prior to fish harvest (Burr et al. 2012; Davidson et al. 2014; Houle et al. 2011; Schrader et al. 2005; Schram et al. 2016; Summerfelt et al. 2015). While depuration systems are an effective means to remediate off-flavor from fish, their necessity increases the capital investment of fish production facilities (B. Vinci, The Conservation Fund Freshwater Institute, personal communication) and adds costs related to fish weight loss resulting from feed deprivation (Burr et al. 2012), energy and oxygen use, and labor associated with fish transport between culture systems.

The lack of success in mitigating off-flavor problems within aquaculture systems that reuse water could be due, in part, to biofilm proliferation throughout unit processes, inside of the pipes, and other hard-to-reach areas that cannot be effectively cleaned. Davidson et al. (2014) demonstrated that the presence of high-surface-area water aeration media in partial reuse depuration systems shielded biofilms from complete disinfection with hydrogen peroxide, thereby resulting in slower remediation of off-flavor levels in Atlantic salmon (*Salmo salar*) fillets. The authors concluded that depuration system designs should exclude high-surface-area media that are difficult to effectively clean and disinfect. However, removal of aeration media from gas exchange columns is not an effective solution in primary fish production systems that reuse water. Therefore, biofilm-management strategies that have

a broad ranging effect throughout reuse systems, including confined areas that cannot be effectively cleaned, need to be considered.

Snails are generally considered a biosecurity threat to fish production because they can carry disease and/or act as intermediate hosts in the life cycle of certain fish parasites (Clausen et al. 2012; Spall and Summerfelt 1970; Terhune et al. 2003; Venable 1998). However, native freshwater snails (*Physa gyrina*) have inadvertently propagated within onsite aquaculture systems without negative effects to fish health and have anecdotally demonstrated potential advantages for fish production. Interestingly, the fillets of market-size (4–5 kg) Atlantic salmon cultured onsite in a semi-commercial-scale RAS were found to have undetectable off-flavor before depuration procedures were applied when *Physa gyrina* were present within various unit processes of the system. Fish production staff hypothesized that *Physa gyrina* may have grazed and managed microbial biofilms that included off-flavor-producing bacteria. Certain snail species are used in small aquaria as cleaner organisms (Lukhaup and Pekny 2015), and snail mucus is known to have antimicrobial properties (Iguchi, Aikawar, and Matsumato 1982; Kubota et al. 1985). However, the symbiotic use of snails in water reuse aquaculture systems used for food fish production has not been reported. To this end, a study was conducted to evaluate the effect of stocking native freshwater snails, *Physa gyrina* on: (1) microbial biofilm and bacterial abundance; and (2) off-flavor producing bacteria and the resultant compounds, geosmin and MIB, while culturing rainbow trout (*Oncorhynchus mykiss*) in replicate water reuse systems.

Materials and methods

Experimental design

Eight experimental-scale partial reuse systems were used for the 3-month study, including four stocked with snails and four without. The system design included a 500-L circular culture tank, a 1/8 HP magnetic pump (Model MD-55RLT, Iwaki Co. Ltd., Tokyo, Japan) to circulate the water, and a 189-L aeration sump in which recycled water trickled through a spray bar to provide ambient gas exchange (Figure 1). Prior to the study, each culture system was cleaned and disinfected by recirculating a hydrogen peroxide solution (250 mg/L) for one hour. During the study, each system received a continuous spring water flow (3.4 ± 0.02 L/min), calibrated two to three times weekly to maintain similar dilution rates among replicates. An average system hydraulic retention time of approximately 2.5 hours was maintained, which resulted in a mean feed loading rate of 0.018 kg feed/m^3 daily makeup water. Effluent flowed through the bottom center drain standpipes of each tank, which were briefly removed several times per week to clear accumulating solids. Fish culture tanks were brushed twice weekly; however, aeration sumps with and without snails were not brushed or cleaned to evaluate the effect of snails.

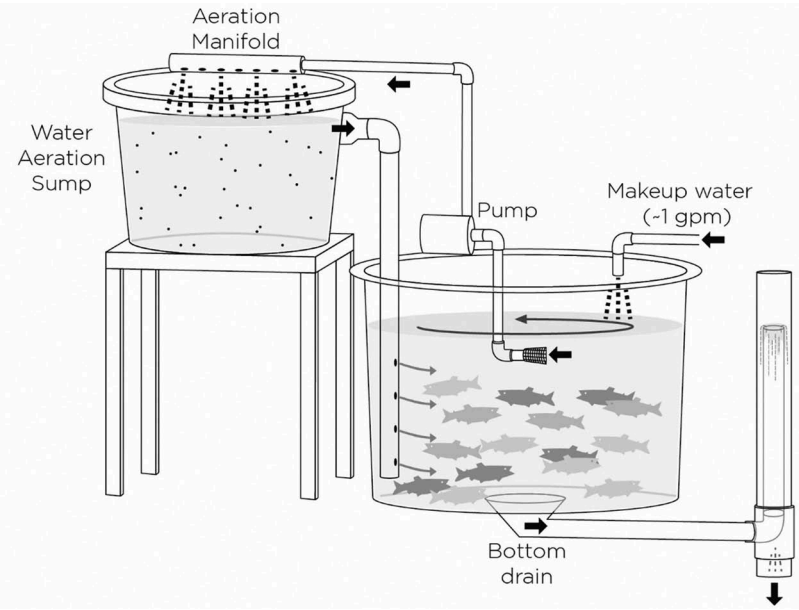


Figure 1. Water flow and process design of a replicate experimental partial reuse system used for the study (courtesy Kata Sharrer, TCFFI Engineering Services). Black dots within aeration sumps denote snails.

Rainbow trout

Rainbow trout were received as fertilized, eyed-eggs (Troutlodge Inc., Bonney Lake, Washington), hatched onsite, and cultured in flow-through tanks. To begin, each reuse system was randomly stocked with 30 rainbow trout (111 ± 1 g). Each day, rainbow trout were hand-fed equal weighed amounts of a 42/16 (protein/fat) trout diet (Zeigler Brothers Inc., Gardners, Pennsylvania). Daily feed amounts ranged from 50–175 g per tank/day over the duration of the study. Mean rainbow trout weight was calculated by bulk weighing the entire population of each tank at the beginning and end of the study; total weight was divided by number of fish per tank. Replicate weights within treatment were averaged for statistical comparison (n = 4). Thermal growth coefficient (TGC) was calculated as follows:

$$\mathrm{TGC} = \left(\mathrm{End\ Weight}^{1/3} - \mathrm{Start\ Weight}^{1/3}\right) / \left((\mathrm{Days\ Between} * \mathrm{Average\ Temperature}) * 1000\right)$$

Mortalities were removed and recorded daily to assess cumulative survival. During the first week, a few mortalities occurred from handling stress, and several fish were lost from jumping out of tanks. Jump screens were secured, and mortalities/losses were replaced with extra trout from the original population to maintain balanced fish numbers among replicates.

Snails

Physa gyrina, commonly known as tadpole or pond snails, were obtained from a freshwater spring that provides source water to our facility in Shepherdstown, West Virginia, and from an effluent discharge location where they had propagated. A total of 100 snails were stocked into each water aeration sump of the four treatment systems. Snails were intentionally isolated in aeration sumps due to concern that rainbow trout might prey upon the snails if comingled. Snails were stocked several days after trout to allow biofilm to accumulate as a food source. Snail size varied; therefore, *Physa gyrina* were distributed among replicate systems based on weight, whereas similar numbers of "large" snails (0.23 ± 0.03 g) and "small" snails (0.044 ± 0.001 g) snails were stocked into each system. The bottoms of aeration tanks housing snails were siphoned two to three times weekly to remove snail feces and dead snails. Deceased snails were counted and replaced with healthy snails.

Water quality sampling and analyses

Water samples were collected weekly from each fish culture tank and tested onsite according to methods described in APHA (2012) and Hach (2003) (Table 1). Part of the total phosphorous analysis was contracted with REI Consultants, Inc. (Beaver, West Virginia; Table 1). Coinciding makeup water quality data assessed during monthly NPDES events is reported for available parameters. Otherwise, a concentration range is reported as background based on unpublished data from other onsite studies.

Table 1. Water quality parameters evaluated and methodologies for testing.

Parameter	Method of analysis
Carbonaceous biochemical-oxygen demand	Standard Methods 5210B—5-day test (no sample prefiltration), Model 58 YSI BOD Probe 5905
Dissolved carbon dioxide	Hach Method 8233—Sodium hydroxide burette titration (pH endpoint) Accumet AB150
Dissolved oxygen	Hach HQ40d with handheld LDO101 probe (Hach Company, Loveland, Colorado)
Heterotrophic bacteria	Hach Method 8242—Membrane filtration, m-TGE broth with TTC Indicator. Fisher Isotemp Incubator #516D
pH	Accumet AB150 benchtop meter
Temperature	Hach HQ40d with handheld LDO101 probe (Hach Company)
Total alkalinity	Standard Methods 2320—Sulfuric acid digital titration (pH endpoint) Accumet AB150
Total ammonia nitrogen	Hach Method 8038—USEPA Nessler. DR 2700, 4000, and 6000
Total phosphorus	Hach Method 8190—Acid Persulfate Digestion (on-site); Method SM 4500-P BE-1999 (REIC, Inc., Beaver, West Virginia)
Total suspended solids	Standard Methods 2540D—Dried at 103°C–105°C
Total solids	Standard Methods 2540B—Dried at 103°C–105°C, Thelco Oven #6540, Mettler Toledo #AE240 and #PM30K

Biofilm and bacteria sampling

Systems were partially drained to facilitate biofilm collection for bacteria sampling and estimation of biofilm abundance via total solids testing (Table 1). For each of these sampling events, biofilm within a rectangular template (78 cm^2) was scraped from three common locations of each aeration sump (total area 233 cm^2), rinsed into a sterile vial with distilled water, and pooled per tank. Assessments of *Streptomyces* spp. abundance and presence of geosmin-producing bacteria were carried out for water and biofilm samples collected from each reuse system at the end of the study. Water was collected from a common location of each fish culture tank. Each of these collection procedures was carried out for individual tanks and analyzed as such. Means were calculated by averaging results from individual tanks within treatment ($n = 4$). Filter papers were placed inside of a sterile housing atop a perforated platform positioned above a 1-L Erlenmeyer flask. A connected diaphragm-air compressor/vacuum pump (Model DOA-9704-AA, Gast Manufacturing Inc., Carlstadt, New Jersey) was used to suction water and biofilm-laden samples through the filters. A pressure differential of 34–51 kPa was exerted during the suction process (Standard Methods 922B.1. f). Each water sample was vigorously mixed prior to filtration, and 250 mL was suctioned through each of two sterile 0.2 μm, 47 mm cellulose nitrate filters (Sartorious™, Thermo Fischer Scientific, Waltham, Massachusetts). Each biofilm sample was mixed vigorously, and portions of each were suctioned through three sterile 0.45 μm, 47 mm S-Pak filter papers (EMD Millipore, Billerica, Massachusetts). Processed biofilm sample volumes varied from 7–50 mL depending on amount of sample that could be vacuumed through the filters. Following filtration, filters were folded in half with tweezers to protect the surface, wrapped in aluminum foil, and held at approximately −10°C for 6 days. Filters were placed between ice packs within an insulated container and shipped to the University of Copenhagen, Denmark, for quantitative PCR (qPCR) analysis. Prior to analysis, triplicates were created by cutting each filter paper into thirds. For detection and quantification of *Streptomyces* cells, filter pieces were treated with 3-fold 20 min freeze–thaw cycles (−70°C to 65°C) to increase cell wall destruction and improve DNA extraction using the PowerWater kit by Qiagen (Venlo, Netherlands). *Streptomyces* abundance was determined by qPCR, combining TaqMan probe and primer technology (Applied Biosystems, Foster City, California), according to Rintala and Nevalainen (2006) and Lylloff et al. (2012). *Streptomyces* abundance data collected from the triplicate filters was averaged per tank; these data were then averaged within treatment ($n = 4$) to calculate a grand mean. In addition, presence of the geosmin synthase gene (geoA) in the extracted DNA was examined using the CycFW and CycRW primers and PCR protocol by Ludwig et al. (2007). Purified PCR products

were cloned into pGEM®-T Easy vector (Promega Life Sciences, Madison, Wisconsin), and subsequently randomly selected clones were sequenced as described by Jørgensen, Podduturi, and Burford (2016). NCBI BLAST tool was used for identification of microorganisms carrying the geosmin synthase gene from the sequence data.

Off-flavor sampling and analysis

Replicate samples of rainbow trout fillets were collected three times during the study at approximately monthly intervals for analysis of off-flavor compounds, geosmin and MIB. Five randomly selected trout from each reuse system were humanely euthanized and filleted for each sampling event. The anterior portion of skinless fillets was vacuum sealed and frozen in preparation for shipment for analysis. Water samples were also collected; unfortunately, challenges occurred (shipping and technical) that prevented accurate reporting of off-flavor in water; therefore, these data are not included. A modified method of Lloyd and Grimm (1999) was used for off-flavor quantification in trout fillets. Samples consisted of 20 g of trout fillet, finely chopped and placed in a glass container, and then spiked with 5 µl of a 10-ppm solution of the internal standard, decahydro-1-naphthol (50 ng). The sample was then heated for 3 min in a microwave while purging with 80 ml/min of N_2. The effluent was transferred via glass tubing to a receiving vessel (20 ml graduated cylinder) in a chilled water bath held at 0°C. The condensed water was brought up to a total volume of 12 ml using deionized water used to rinse the transfer line. This sample was then divided into 6 ml aliquots, each placed into a 10-ml vial. Three grams of NaCl were added to the vial and then sealed with a crimp cap fitted with a Viton septum. Samples were loaded on a CTC SPME autosampler (Leap Technologies, Morrisville, North Carolina). Individual samples were first heated to 65°C and exposed to the SPME fiber for a 15-min adsorption period while undergoing vigorous agitation. The autosampler was equipped with a 1-cm-long, divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco, Inc. Bellefonte, Pennsylvania). The fiber was withdrawn from the sample and desorbed at 280°C for 2 min in the injection port of an HP6890 GC equipped with a 5973-mass selective detector (Hewlett Packard, Palo Alto, California). The injection port was operated in pulsed splitless mode and fitted with a 0.7 mm ID injection liner. Head pressure was set to 50 psi of Helium for the first minute, and then to a constant velocity of 40 cm/sec for the remainder of the GC run. The GC oven was held at 40°C for 2 min then increased at a rate of 10°C/min to 200°C then 50°C/min to 300°C and held for a 30-min run. The quadrupole mass spectrometer was operated in selected ion monitoring (SIM) mode, and geosmin was quantified on m/z 112 with qualifier ions of m/z 125 and 182. MIB was quantified using m/z 95 with m/z 135 and 168 as qualifiers.

Snail salinity tolerance

After the main study, a separate month-long trial was conducted to evaluate the tolerance of *Physa gyrina* to increased salinity. Salt is periodically added within onsite reuse systems to produce short-term salinity ≤ 4.0 ppt as a fish health treatment for opportunistic bacterial and fungal infections; therefore, this trial sought to determine the salinity tolerance of *Physa gyrina* from 0.2–4.0 ppt. Snails of common size (8–11 mm wide) were collected from the spring and facility discharge and stocked into 20 plastic containers (500 mL; 10 snails/unit), including four containers maintained with each of the following salinity treatments: 0.2, 1, 2, 3, and 4 ppt. Salt concentrations were created by mixing predetermined amounts of Diamond Crystal Solar Naturals Salt Crystals (Cargill Inc., Minneapolis, Minnesota) with onsite spring water (0.2 ppt) to achieve the respective salinities. Saline water was replaced with freshwater for all treatments on a weekly basis, thus treatment containers rotated from saline treatment to freshwater to simulate periodic presence/absence of salinity as is common with a salt treatment regimen in water reuse systems. Containers were placed at the bottom of a 500-L tank, where cool (13–14°C) spring water circulated around the base for ambient temperature control. Air lines extending from a common air pump were inserted into the lids of each container for aeration, and equal weighed amounts of store-bought lettuce were provided ad libitum as a food source. Salinity (YSI Model 30/10, YSI Inc., Yellow Springs, Ohio), dissolved oxygen (Hach HQ40d, Hach Company, Loveland, Colorado), and temperature were measured three times weekly, and water was changed twice weekly. During water replacement events, mortalities and egg masses were counted to assess cumulative survival and reproduction rates respectively. Egg masses were removed during water changes to assess further reproduction.

Statistical analyses

Student's *t*-test was utilized to compare response variables between treatments, including separate analysis of geosmin concentrations from fish samples collected each month. Each data set was analyzed for normality using a Shapiro-Wilk test. The Mann-Whitney U test was utilized if data were not normally distributed. Linear regression analysis was used to quantify the relationship between geosmin in trout fillets with time. A probability level of 0.05 was used to determine significance. Statistical analyses were carried out using SYSTAT Version 13 (Systat Software, San Jose, California).

Results

Biofilm abundance

A visible difference in biofilm was evident in aeration sumps with and without snails for most of the study. Sumps containing snails had little to no biofilm, while the submerged surfaces of sumps without snails were coated with biofilm (Figure 2). Total biofilm solids per sample from reuse systems with and without snails were 7 ± 4 and 270 ± 37 mg dry weight respectively; total solids per cm^2 of scraped area were 0.03 ± 0.02 and 1.16 ± 0.16 mg/cm^2 for systems with and without snails respectively (P = 0.006).

Heterotrophic bacteria

Mean heterotrophic bacteria counts in the culture tank water were lower (P = 0.043) in reuse systems with snails versus those without snails, i.e., 311 ± 87 vs. 1502 ± 326 cfu/mL respectively. The distribution of mean heterotrophic bacteria data was distinctly different between treatments, except for one outlier that indicated consistently low bacteria counts in a replicate system without snails, i.e., 526 cfu/mL. This outlier was maintained in the data set and therefore resulted in a skewed distribution, thus the nonparametric Mann-Whitney U test was utilized to compare heterotrophic bacteria counts between treatments. Mean water quality levels were statistically similar between treatments for all other tested parameters (Table 2).

Streptomyces abundance and identification

Average *Streptomyces* spp. abundance in filtered biofilm samples was approximately 1 log lower in reuse systems with snails compared to the control, 4.1×10^4 and 1.2×10^5 cells/100 mL respectively (Figure 3); however, due to variability

Figure 2. Biofilm abundance in aeration sumps (No Snails [L]) and Snails [R]) near the end of the trial. Photos courtesy Kata Sharrer, TCFFI Engineering Services.

Table 2. Water quality concentrations (mean ± 1 SEM; n = 4) assessed in systems with and without snails (mg/L unless noted otherwise).

Parameter	No snails	Snails	[a]Makeup water
Carbonaceous biochemical- oxygen demand	1.35 ± 0.02	1.26 ± 0.04	0.22 ± 0.18
Dissolved carbon dioxide	11 ± 1	11 ± 1	15– 20
Dissolved oxygen	8.7 ± 0.1	8.7 ± 0.2	13.0– 16.0
[b]Heterotrophic bacteria (cfu mL^{-1})	1502 ± 326	311 ± 87	10– 20
pH	7.85 ± 0.05	7.80 ± 0.05	7.3– 7.5
Temperature (°C)	14.6 ± 0.05	14.6 ± 0.03	13.5 ± 0.3
Total alkalinity	267 ± 1	263 ± 3	260– 270
Total ammonia nitrogen	0.27 ± 0.01	0.28 ± 0.01	0.02 ± 0.01
Total phosphorous	0.134 ± 0.003	0.133 ± 0.004	0.020 ± 0.010
Total suspended solids	1.2 ± 0.1	1.0 ± 0.1	0.4 ± 0.1

[a]Coinciding makeup water quality data assessed during monthly NPDES sampling events reported for available parameters; otherwise, a concentration range is shown based on unpublished data from other onsite studies.
[b]Indicates statistically significant difference between treatments.

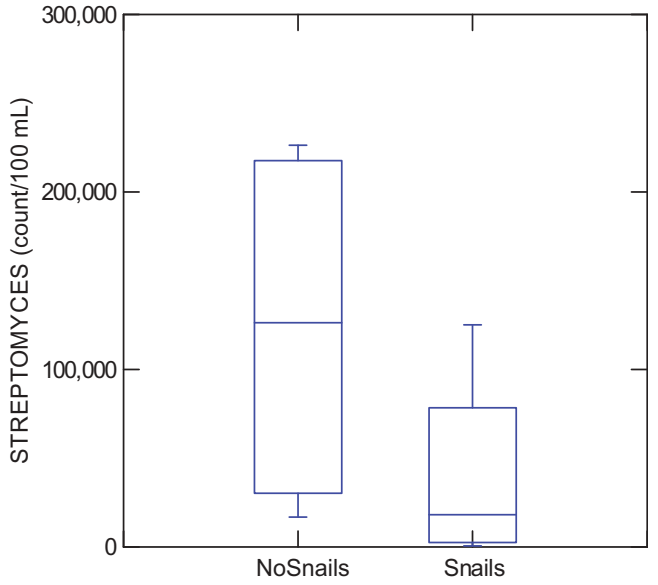


Figure 3. *Streptomyces* spp. abundance in filtered biofilm samples collected from systems with and without snails.

within treatments, a significant difference was not detected. *Streptomyces* abundance in water samples was not affected by treatment. *Streptomyces* abundance in water samples collected from reuse systems with and without snails was 515 ± 55 and 425 ± 130 cells/100 mL. PCR results for the geosmin synthase gene geoA confirmed the presence of geosmin-producing organisms in water and biosolids samples from both treatments. Clones of geoA and their taxonomic affiliates indicated the presence of various groups of geosmin producers, including: actinobacteria, myxobacteria, and cyanobacteria (Table 3). Myxobacteria seemed to be

Table 3. List of geosmin-producing organisms present in reuse systems, based on molecular cloning and sequencing of geosmin synthase (geoA) PCR products.

		NCBI—blastx output		
Source DNA	Clone ID	Similarity suggestion	% identity	Accession no.
Water from systems with snails	FI-6-4	*Streptomyces*[a]	69	WP_009067911.1
	FI-6-6	*Streptomyces griseoflavus*[a]	64	KOG59425.1
	FI-6-9	*Archangium gephyra*[b]	82	WP_047855443.1
	FI-6-10	*Nannocystis exedens*[b]	73	SFE34769.1
Biosolids from systems with snails	FI-31-3	*Sorangium cellulosum*[b]	74	WP_061610909.1
	FI-31-5	*Sorangium cellulosum*[b]	74	WP_061610909.1
	FI-31-7	*Chondromyces apiculatus*[b]	87	EYF04694.1
	FI-31-8	*Sorangium cellulosum*[b]	76	WP_061610909.1
Water from systems without snails	FI-7-1	*Sorangium cellulosum*[b]	76	WP_061610909.1
	FI-7-3	*Cystobacter fuscus*[b]	83	EPX58447.1
	FI-7-4	*Nocardia salmonicida*[a]	67	WP_062982054.1
	FI-7-5	*Sorangium cellulosum*[b]	74	WP_061610909.1
Biosolids from systems without snails	FI-23-1	*Chondromyces crocatus*[b]	82	WP_050434199.1
	FI-23-2	*Oscillatoria* sp. 327/2[c]	94	AIZ06055.1
	FI-23-3	*Nostoc* sp. UK4[c]	72	AIZ06052.1
	FI-23-4	*Hyalangium minutum*[b]	91	WP_044199666.1
	FI-23-5	*Stigmatella erecta*[b]	76	WP_093525208.1
	FI-23-6	*Hyalangium minutum*[b]	92	WP_044199666.1

[a]Actinobacteria; [b]Myxobacteria; [c]Cyanobacteria.

abundant in the system, since myxobacterial sequences were detected in all tested samples. Among the geoA clones, actinobacteria (*Streptomyces* and *Nocardia*) were mostly present in the water phase and were minimally detected in biosolids. The biosolids from the snail treatment were dominated by myxobacteria, whereas both myxobacteria and cyanobacteria were present in biosolid samples from systems without snails.

Off-flavor

Geosmin levels in rainbow trout fillets were not statistically different between treatments but were generally lower in trout fillets from reuse systems with snails (Figure 4). Concentrations measured in trout fillets from reuse systems with and without snails increased linearly (R^2 = 0.941, 0.992 respectively) over the study duration. Geosmin levels accumulated in trout fillets to levels of approximately 350 to 1000 ng/kg, within reported limits of human detection for salmonids (Burr et al. 2012; Robertson et al. 2005). MIB was not detected in trout fillets from either treatment (i.e., ions indicative of MIB were < detection levels of 0.005 ppb) but was measured in the standards.

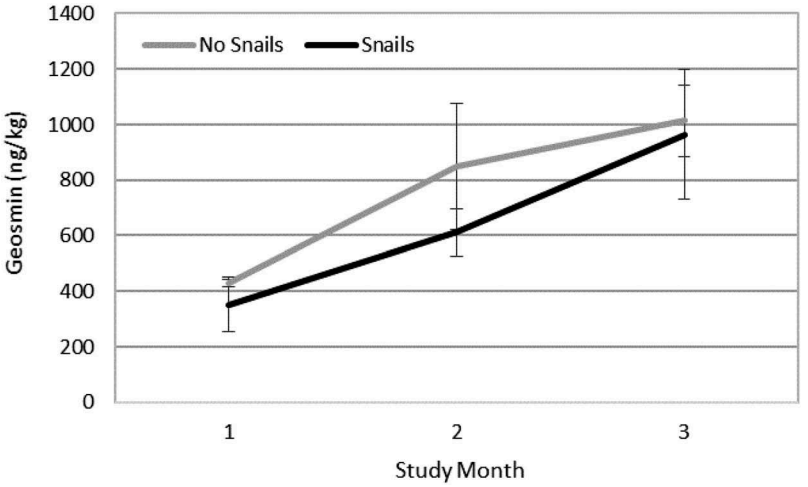


Figure 4. Geosmin concentrations measured in rainbow trout fillets at approximately monthly intervals for each treatment.

Trout performance

Rainbow trout growth was not affected by the presence of snails. Mean thermal growth coefficient calculated for trout cultured within each treatment was 2.1 ± < 0.1. At study's end, mean rainbow trout weight in reuse systems with and without snails was 355 ± 3 and 356 ± 3 g respectively. During the first week of the study, a few mortalities (6 ± 2 per tank) occurred independent of treatment due to fish jumping out of tanks and from handling stress. Thereafter, only one mortality was removed from a replicate system containing snails and no mortalities were removed from the control treatment; thus, rainbow trout survival was not affected by the presence of snails.

Snail performance

Snails rapidly proliferated in the treatment systems. Within the first month of the study, transparent embryo-containing jelly masses were observed (Figure 5), and soon after, tiny snails were noticed in each treatment tank. Snail mortality occurred regularly in each treatment system, and these snails were replaced to maintain balanced replicates. During the supplemental salinity tolerance trial, no significant differences were detected in snail survival at 0–4 ppt salinity. However, significant snail mortality occurred for each treatment; snail survival among treatments was 41 ± 5%. In addition, salinity did not affect snail reproduction as measured by the cumulative number of egg masses counted per experimental unit per treatment, i.e. 5–6 jelly masses per container.

Figure 5. *Physa gyrina* snail egg masses and juvenile snails observed during the experiment (courtesy Kata Sharrer, TCCFFI Engineering Services).

Discussion

Physa gyrina have a wide geographical range spanning most freshwater habitats of North America (Dillon et al. 2013), which can be attributed to their adaptive feeding behavior. *Physa gyrina* are known to consume a variety of materials, including biofilm (Palsdottir and Bishop 1997), detritus, diatoms, filamentous algae, fungi, living animal and plant vascular tissue, and water molds (Brown 1982; Dewitt 1955; Dillon et al. 2013). During this study, *Physa gyrina* consumed available biofilm within the aeration sumps of treatment systems (Figure 2). The propensity for *Physa gyrina* to control microbial biofilms in the experimental reuse systems is promising because biofilms sampled from recirculation aquaculture systems have been found to include pathogenic bacteria (King et al. 2004, 2006, 2008) and harmful off-flavor-producing bacteria (Schrader and Summerfelt 2010).

Despite thorough removal of microbial biofilms by snails during the present study, off-flavor concentrations in rainbow trout fillets were not significantly reduced. First, it should be noted that MIB was not detected in fish samples from either treatment. This result is not unusual because MIB is typically low or absent in onsite water and fish samples (Schrader, Davidson, and Summerfelt 2013; Schrader and Summerfelt 2010). Geosmin

was measured at typical concentrations in trout fillets from both treatments, but there was no significant effect of snails on geosmin levels. Nevertheless, the slightly slower (albeit nonsignificant) rate of geosmin accumulation from Month 1 to 2 in fish collected from systems with snails (Figure 4) is noteworthy when considering the dramatic reduction in biofilm by snails (Figure 2) and the lower abundance of off-flavor-producing *Streptomyces* (Figure 3) found in minimally remaining biofilm sampled from these aeration sumps. These combined responses align with reduced off-flavor in water and fish fillets. *Streptomyces* bacteria were the focus of this study because they have been reported as common off-flavor-producing bacteria in recirculation systems, and most species produce geosmin and/or MIB (Schrader et al. 2010; Sugiura and Nakano 2000). Other geosmin-producing bacteria species were present in the experimental systems. The present cloning of geoA PCR products supports that myxobacteria were abundant in both water and biofilm of the tanks and that *Streptomyces* were more prevalent in the water. Myxobacteria have been documented as geosmin-producers (Dickshat et al. 2005; Yamamoto, Sakata, Tanaka 2000), but only a few prior studies have identified these bacteria as producers of odorous compounds in fish production systems (Auffret et al. 2013; Lukassen et al. 2017). Other microorganism phyla and a variety of newly identified off-flavor compounds have also been reported in fish production systems (Mahmoud and Buettner 2016, 2017; Podduturi et al. 2017), thus future research involving the effect of snails on off-flavor may consider focusing on a range of off-flavor-producing bacteria and compounds.

The lack of profound difference in geosmin between treatments might be explained by the potential presence of off-flavor-producing bacteria in other areas of the reuse systems. *Physa gyrina* were confined to the water aeration sumps, and although the snails thoroughly removed biofilm at this location, microbial growth still occurred in other areas where snails were not present, including the fish culture tanks and inside of pumps and piping. Follow-up research evaluating effects on off-flavor with snails proliferated throughout partial reuse systems and in RAS with relatively low water exchange would be informative. Several studies have noted increased microbial selection and stabilization in systems with longer retention times, particularly noting improved fish health and possible inhibition of pathogenic bacteria (Attramadal et al. 2012, 2014; De Schryver and Vadstein 2014). To our knowledge, the effect of microbial selection and maturity on off-flavor-producing bacteria has not been studied; thus it would be interesting to assess how snails might influence microbial selection in systems with varying water exchange rates. In addition, the metabolism of geosmin and MIB by *Physa gyrina* is unknown. It is unclear whether *Physa gyrina* are capable of biotransforming off-flavor compounds to a nonodorous chemical form, or if these compounds are excreted back into the water and/or possibly solubilized

from snail fecal matter. During a preliminary evaluation, geosmin was detected in snail tissue, but sufficient numbers of snails were not available during the study to produce ample tissue for analysis; therefore, more research in this area may be warranted.

Although a statistical effect of snails on off-flavor was not found during this study, the snails produced other effects that could be advantageous for cohabitation in water reuse systems. The ability of *Physa gyrina* to dramatically reduce biofilm growth indicates that these mollusks could act as cleaner organisms in reuse systems. Other snail species are commonly introduced to small aquaria with similar intent (Lukhaup and Pekny 2015). Much of the biofilm removal likely resulted from snail grazing; however, biofilm abundance could also have been influenced by the potential antimicrobial properties of snail mucus (Iguchi, Aikawar, and Matsumato 1982; Kubota et al. 1985). Regardless, the biofilm-limiting ability demonstrated by *Physa gyrina* could lead to reduced labor associated with brushing and cleaning submerged surfaces of tanks and unit processes in aquaculture systems. Furthermore, the efficiency of biofilm removal by the snails resulted in a significant reduction in the total heterotrophic bacteria load in the culture water. These results may indicate the potential for snails to control opportunistic and obligate pathogenic bacteria populations. Reduction of heterotrophic bacteria counts in systems that reuse water is a relatively profound result. Microbial biofilms can establish inside of pipes and on submerged surfaces of unit processes throughout water reuse systems; therefore, it is difficult to significantly influence these microbial populations. During other onsite trials, heterotrophic bacteria counts were not significantly reduced even when applying powerful oxidants such as ozone (Davidson et al. 2011; Good et al. 2017) or peracetic acid (authors' unpublished data). Davidson et al. (2011) described trends for bacterial reduction by ozone, but the variability of bacterial counts among replicate RAS limited detection of significant differences. The variability of oxidant impact on microbial populations in water reuse systems could, in part, be related to their effectiveness being limited to a section of the water recycle loop where they are applied and the rapid dissipation of residual concentrations. On the other hand, snails could populate across an expansive submerged surface area of water reuse systems, including inside of pipes, and therefore could provide a wider ranging effect on microbial populations.

A potential drawback of cohabiting *Physa gyrina* in aquaculture systems is the uncertainty of survivability and recruitment of progeny under various conditions. Snail mortality occurred regularly throughout the trial, but the cause was unclear. It was difficult to accurately assess the extent of the mortality due to rapid reproduction rates and occasional snail escapement from the water aeration sumps. The authors hypothesize that mortality occurred, in part, as snails rapidly grazed available biofilm and exhausted their food supply. Additional research may

be required to understand appropriate snail stocking density per unit surface area. In addition, larger snails removed as mortalities could have perished due to age; the average life expectancy of *Physa gyrina* has been reported to be just 12–13 months under natural conditions (DeWitt 1954). Nevertheless, *Physa gyrina* demonstrated unabated fecundity and seemed to naturally maintain carrying capacity with the emergence of juvenile snails. More than likely, *Physa gyrina* mortality was not related to water quality during this study. Due to continuous spring water addition and relatively short hydraulic retention time, water quality was similar to onsite spring water where *Physa gyrina* were collected; therefore, it is reasonable to assume that these conditions were suitable. In addition, results of the supplementary salinity trial suggest that *Physa gyrina* are tolerant to salinities from near 0 ppt to the maximum tested salinity of 4 ppt. These findings resemble those of Kefford and Nugegoda (2005), who found no evidence of a critical salinity threshold for a phylogenic relative, *Physa acuta*. Based on the results from the supplementary salinity trail, *Physa gyrina* would likely tolerate short-term salt treatments used to aid fish health in water reuse systems.

Although the introduction of snails in water reuse aquaculture systems appears to offer certain advantages, caution should be taken before comingling snails. *Physa gyrina* have been implicated for disrupting nitrification in waste water treatment plants due to grazing of nitrifying biofilms (Palsdottir and Bishop 1997). Snails could also clog the orifices of spray bar inlets, drum filter spray nozzles, pump intake screens, or other important components of reuse systems. Some fish production system designs may be more appropriate for incorporating snails, such as those that utilize moving biofilter media that are less liable for snail attachment, e.g., fluidized sand biofilters. It should also be noted that Rakocy, Masser, and Losordo (2006) described snails as pests that consume roots and important nitrifying bacteria in aquaponics systems. Furthermore, snails can carry disease and/or act as intermediate hosts in the life cycle of certain fish parasites (Clausen et al. 2012; Spall and Summerfelt 1970; Terhune et al. 2003; Venable 1998). Therefore, biosecurity risks should be stringently considered before introducing snails into fish production systems. The snails used in the present study were not prescreened for listed fish pathogens but were anecdotally considered to be innocuous because *Physa gyrina* are inherent to the onsite spring and have periodically coexisted in our fish culture systems without obligate fish pathogen or major parasitic outbreaks. The general good growth, health, and survival of rainbow trout during the present study supports this hypothesis. Other practical considerations are also required to fully demonstrate the feasibility of stocking snails in water reuse systems. For example, more research is required to understand the propensity for rainbow trout and/or other aquaculture species to consume snails that are cohabited with fish in culture tanks. This aspect is not so simple to study because snails can escape culture vessels through drains, pipes, and other outlets, but experimentation

could be carried out with fish and snails held together at low densities in closed aquaria. In addition, the appropriateness of using snail species other than *Physa gyrina* may need to be considered based on local availability, grazing behavior (food preference) of available snails, and biosecurity concerns. Use of endemic snails, as was demonstrated during the present study, is encouraged. Lastly, maintenance of a consistent supply of snails in separate, onsite aquaria may need to be considered for sustained cohabitation of snails in water reuse systems. Semiregular stocking and replenishment of snails may be required to account for snail mortality and to maintain large enough populations to control microbial biofilms.

Conclusions

The present trial was a proof-of-concept study that indicated potential microbial advantages for integrating *Physa gyrina* snails and rainbow trout in water reuse aquaculture systems. The introduction of snails substantially reduced biofilm abundance and total heterotrophic bacteria counts in the culture water. Off-flavor concentrations were not significantly reduced in trout fillets; however, geosmin was generally lower in systems with snails, and Streptomyces bacteria, which are typically associated with off-flavor production, were less abundant in biofilm. Based on thorough biofilm control and reduction in heterotrophic populations caused by *Physa gyrina*, the authors hypothesize that these snails could provide control over biofilm and microbial populations throughout water reuse systems, including potential pathogenic bacteria. Future research evaluating pathogen control by snails may be important in evaluating their symbiotic use in water reuse systems. Most importantly, follow-up research should investigate the microbial effects of *Physa gyrina* or other select snails when snails are permitted to proliferate throughout the pipes and unit processes of reuse systems, while assessing associated challenges. A variety of other practical considerations and questions need to be researched and resolved before this approach can be confidently adopted, such as: understanding the effects of snails on the function of system components, biosecurity challenges, snail carrying capacity, supply of snails, and whether certain species of snails with commercial value can be used.

Acknowledgments

Special thanks to Karen Schroyer, Susan Glenn, Brianna Taylor, Christina Russell, and Natalie Redman for water chemistry analyses; to student technicians Michael "Wells" Larson, Heather Dailey, and Chloe Raines for fish husbandry and technical assistance; to Kata Sharrer for photography and drawings; to Christine Lepine for literature review and assistance with manuscript preparation; and to Jennifer Aalhus, Manuel Juarez, and Eric Ruan for analytical work conducted at the Lacombe Research Centre (Alberta, Canada).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the USDA Agricultural Research Service under Agreement Numbers 59-1930-5-510 and 59-8082-5-001. Opinions, conclusions, and recommendations are of the authors and do not necessarily reflect the views of the USDA. All experimental protocols and methods complied with Animal Welfare Act (9CFR) requirements and were approved by the Freshwater Institute's Institutional Animal Care and Use Committee.

ORCID

John Davidson http://orcid.org/0000-0002-6246-798X
Carol Zygar Plautz http://orcid.org/0000-0003-1903-5935
Niels O.G. Jørgensen http://orcid.org/0000-0002-3554-6906

References

Acuña-Rubio, S. 2002. "Evaluation of methods for geosmin and MIB removal from recirculating aquaculture systems." Master's Thesis. University of California Davis. 10.1044/1059-0889(2002/er01)

Aly, S. M., Y. A.-G. Ahmed, -A.-A.-A. Ghareeb, and M. F. Mohamed. 2008. Studies on Bacillus subtilis and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge fish infections. *Fish and Shellfish Immunology* 25:128–36. doi:10.1016/j.fsi.2008.03.013.

American Public Health Association (APHA). 2012. *Standard methods for the examination of water and wastewater*. 22nd ed. Washington, District of Columbia: American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF).

Antonopoulou, M., E. Evgenidou, D. Lambropoulou, and I. Konstantinou. 2014. A review on advanced oxidation processes for the removal of taste and odor compounds from aqueous media. *Water Research* 53:215–34. doi:10.1016/j.watres.2014.01.028.

Attramadal, K. J. K., I. Salvesen, R. Y. Xue, G. Øie, T. R. Størseth, O. Vadstein, and Y. Olsen. 2012. Recirculation as a possible microbial control strategy in the production of marine larvae. *Aquacultural Engineering* 46:27–39. doi:10.1016/j.aquaeng.2011.10.003.

Attramadal, K. J. K., T. M. Truong, I. Bakke, J. Skjermo, Y. Olsen, and O. Vadstein. 2014. RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. *Aquaculture* 432:483–90. doi:10.1016/j.aquaculture.2014.05.052.

Auffret, M., É. Yergeau, A. Pilote, É. Proulx, D. Proulx, C. W. Greer, G. Vandenberg, and R. Villemur. 2013. Impact of water quality on the bacterial populations and off-flavours in recirculating aquaculture systems. *FEMS Microbiology Ecology* 84:235–47. doi:10.1111/1574-6941.12053.

Azad, I. S., K. M. Shankar, C. V. Mohan, and B. Kalita. 1999. Biofilm vaccine of *Aeromonashydrophila* – Standardization of dose and duration for oral vaccination of carps. *Fish and Shellfish Immunology* 9:519–28. doi:10.1006/fsim.1998.0206.

Brown, K. M. 1982. Resource overlap and competition in pond snails: An experimental analysis. *Ecology* 63:412–22. doi:10.2307/1938959/.

Brown, S. W., and C. E. Boyd. 1982. Off-flavor in channel catfish from commercial ponds. *Transactions of the American Fisheries Society* 111:379–83. doi:10.1577/1548-8659(1982)111<379:OICCFC>2.0.CO;2.

Burr, G. S., W. R. Wolters, K. K. Schrader, and S. T. Summerfelt. 2012. Impact of depuration of earthy-musty off-flavors on fillet quality of Atlantic salmon, *Salmo salar*, cultured in a recirculating aquaculture system. *Aquacultural Engineering* 50:28–36. doi:10.1016/j.aquaeng.2012.03.002.

Cai, W., L. D. L. Fuente, and C. R. Arias. 2013. Biofilm formation by the fish pathogen *Flavobacterium columnare*: Development and parameters affecting surface attachment. *Applied and Environmental Microbiology* 79 (18):5633–42. doi:10.1128/AEM.01192-13.

Chen, S., J. Ling, and J.-P. Blancheton. 2006. Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* 34:179–97. doi:10.1016/j.aquaeng.2005.09.004.

Clausen, J. H., H. Madsen, K. D. Murrell, V. P. Thi, H. N. Manh, K. H. Viet, and A. Dalsgaard. 2012. Relationship between snail population density and infection status of snails and fish with zoonotic trematodes in Vietnamese carp nurseries. *PLOS Neglected Tropical Diseases* 6:e1945. doi:10.1371/journal.pntd.0001945.

Davidson, J., C. Good, C. Welsh, and S. Summerfelt. 2011. The effects of ozone and water exchange rates on water quality and rainbow trout *Oncorhynchus mykiss* performance in replicated water recirculating systems. *Aquacultural Engineering* 44:80–96. doi:10.1016/j.aquaeng.2011.04.001.

Davidson, J., K. Schrader, E. Ruan, B. Swift, J. Aalhus, M. Juarez, W. Wolters, G. Burr, C. Good, and S. T. Summerfelt. 2014. Evaluation of depuration procedures to mitigate the off-flavor compounds geosmin and 2-methylisoborneol from Atlantic salmon *Salmo salar* raised to market-size in recirculating aquaculture systems. *Aquacultural Engineering* 61:27–34. doi:10.1016/j.aquaeng.2014.05.006.

De Schryver, P., and O. Vadstein. 2014. Ecological theory as a foundation to control pathogenic invasion in aquaculture. *The ISME Journal* 8 (12):2360–68. doi:10.1038/ismej.2014.84.

Dewitt, R. M. 1955. The ecology and life history of the pond snail, *Physa gyrina*. *Ecology* 36:40–44. doi:10.2307/1931429.

DeWitt, R. M. 1954. Reproduction, embryonic development, and growth in the pond snail, *Physa gyrina*. *Transactions of the American Microscopical Society* 73:124–37. doi:10.2307/3223750.

Dickshat, J. S., H. B. Bode, T. Mahud, R. Muller, and S. Schulz. 2005. A novel type of geosmin biosynthesis in myxobacteria. *Journal of Organic Chemistry* 70:5174–82. doi:10.1021/jo050449g.

Dillon, R. T. Jr., M. Ashton, M. Kohl, W. Reeves, T. Reeves, T. Smith, T. Stewart, and B. Watson. 2013. "The freshwater gastropods of North America." Accessed April 25, 2018. http://www.fwgna.org

Engle, C. R., G. L. Pounds, and M. Ploeg. 1995. The cost of off-flavor. *Journal of the World Aquaculture Society* 26:297–306. doi:10.1111/j.1749-7345.1995.tb00258.x.

Good, C., J. Davidson, R. I. Earley, J. Styga, and S. Summerfelt. 2017. The effects of ozonation on select waterborne steroid hormones in recirculation aquaculture systems containing sexually mature Atlantic salmon *Salmo salar*.. *Aquacultural Engineering* 79:9–16. doi:10.1016/j.aquaeng.2017.08.004.

Guttman, L., and J. van Rijn. 2008. Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. *Aquaculture* 279:85–91. doi:10.1016/j.aquaculture.2008.03.047.

Hach. 2003. *DR/4000 Spectrophotometer Procedures Manual*. Loveland, CO: Hach Company.

Hagopian, D. S., and J. G. Riley. 1998. A closer look at the bacteriology of nitrification. *Aquacultural Engineering* 18:223–44. doi:10.1016/S0144-8609(98)00032-6.

Hansen, T. R. 2003. Economic impact of off-flavor to the U.S. catfish industry. In *Off-flavors in Aquaculture*, eds. A. M. Rimando, and K. K. Schrader, 13–29. Washington D.C.: American Chemical Society.

Houle, S., K. K. Schrader, N. R. Le François, Y. Comeau, M. Kharoune, S. T. Summerfelt, A. Savoie, and G. W. Vandenberg. 2011. Geosmin causes off-flavour in arctic charr in recirculating aquaculture systems. *Aquaculture Research* 42:360–65. doi:10.1111/j.1365-2109.2010.02630.x.

Howgate, P. 2004. Tainting of farmed fish by geosmin and 2-methyl-iso-borneol: A review of sensory aspects and of uptake/depuration. *Aquaculture* 234:155–81. doi:10.1016/j.aquaculture.2003.09.032.

Iguchi, S. M., T. Aikawar, and J. J. Matsumato. 1982. Antibacterial activity of snail mucus mucin. *Comparative Biochemistry and Physiology - Part A: Comparative Physiology* 72:571–74. doi:10.1016/0300-9629(82)90123-2.

Jørgensen, N. O. G., R. Podduturi, and M. A. Burford. 2016. Relations between abundance of potential geosmin- and 2-MIB-producing organisms and concentrations of these compounds in water from three Australian reservoirs. *Journal of Water Supply: Research and Technology—AQUA* 65:504–13. doi:10.2166/aqua.2016.001.

Kaiser, G. E., and F. W. Wheaton. 1983. Nitrification filters for aquatic culture systems: State of the art. *Journal of the World Aquaculture Society* 14:302–24. doi:10.1111/j.1749-7345.1983.tb00086.x.

Kefford, B. J., and D. Nugegoda. 2005. No evidence for a critical salinity threshold for growth and reproduction in the freshwater snail *Physa acuta*. *Environmental Pollution* 134:77–383. doi:10.1016/j.envpol.2004.09.018.

King, R. K., G. Flick, D. Pierson, S. Smith, G. D. Boardman, and C. W. Coale. 2004. Identification of bacterial pathogens in biofilms of recirculating aquaculture systems. *Journal of Aquatic Food Production Technology* 13 (1):125–33. doi:10.1300/J030v13n01_11.

King, R. K., G. J. Flick Jr., S. A. Smith, M. D. Pierson, G. D. Boardman, and C. W. Coale Jr. 2006. Comparison of bacterial presence in biofilms on different materials commonly found in recirculating aquaculture systems. *Journal of Applied Aquaculture* 18 (1):79–88. doi:10.1300/J028v18n01_05.

King, R. K., G. J. Flick Jr., S. A. Smith, M. D. Pierson, G. D. Boardman, and C. W. Coale Jr. 2008. Response of bacterial biofilms in recirculating aquaculture systems to various sanitizers. *Journal of Applied Aquaculture* 20 (2):79–92. doi:10.1080/10454430802191766.

Klausen, M. M., and O. Grønborg. 2010. Pilot scale testing of advanced oxidation processes for degradation of geosmin and MIB in recirculated aquaculture. *Water Science and Technology: Water Supply* 10:217–25. doi:10.2166/ws.2010.246.

Kubota, Y. W., H. Otsuka, T. Tamiya, T. Suchiya, and J. J. Matsumoto. 1985. Purification and characterization of an antibacterial factor from snail mucus. *Comparative Biochemistry and Physiology C* 82:345–48. doi:10.1016/0742-8413(85)90173-2.

Kuhn, D. D., A. L. Lawrence, G. D. Boardman, S. Patnaik, L. Marsh, and G. J. Flick. 2010. Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 303:28–33. doi:10.1016/j.aquaculture.2010.03.001.

Lloyd, S. W., and C. C. Grimm. 1999. Analysis of 2-methylisoborneol and geosmin in catfish by microwave desorption - solid phase microextraction. *Journal of Agricultural and Food Chemistry* 47:164–69. doi:10.1021/jf980419x.

Ludwig, F., A. Medger, H. Börnick, M. Opitz, K. Lang, M. Göttfert, and I. Röske. 2007. Identification and expression analyses of putative sesquiterpene synthase genes in *Phormidium* sp. and prevalence of geoA-like genes in a drinking water reservoir. *Applied and Environmental Microbiology* 73:6988–93. doi:10.1128/AEM.01197-07.

Lukassen, M. B., A. M. Saunders, P. D. Sindilariu, and J. L. Nielsen. 2017. "Quantification of novel geosmin-producing bacteria in aquaculture systems.". *Aquaculture* 479:304–10. doi:10.1016/j.aquaculture.2017.06.004.

Lukhaup, C., and R. Pekny. 2015. *Invertebrates – Shrimp, crayfish, crabs, and snails in freshwater aquaria*. Ettlingen, Germany: Dahne Verlag.

Lylloff, J. E., M. H. Mogensen, M. A. Burford, L. Schlüter, and N. O. G. Jørgensen. 2012. Detection of aquatic streptomycetes by quantitative PCR for prediction of taste-and-odour episodes in water reservoirs. *Journal of Water Supply: Research and Technology* 61:272–82. doi:10.2166/aqua.2012.006.

Ma, N., G. Luo, H. Tan, L. Li, and X. Wang. 2016. Removal of geosmin and 2-methylisoborneol by bioflocs produced with aquaculture waste. *Aquaculture International* 24:345–56. doi:10.1007/s10499-015-9929-0.

Mahmoud, M. A. A., and A. Buettner. 2016. Characterisation of aroma-active and off-odour compounds in German rainbow trout (*Oncorhynchus mykiss*). Part 1. Case of aquaculture water from earthen-ponds farming. *Food Chemistry* 210:623–30. doi:10.1016/j.foodchem.2016.05.030.

Mahmoud, M. A. A., and A. Buettner. 2017. Characterisation of aroma-active and off-odour compounds in German rainbow trout (*Oncorhynchus mykiss*). Part II. Case of fish meat and skin from earthen-ponds farming. *Food Chemistry* 232:840–49. doi:10.1016/j.foodchem.2016.09.172.

McDowall, B., D. Hoefel, G. Newcombe, C. P. Saint, and L. Ho. 2009. Enhancing the biofiltration of geosmin by seeding sand filter columns with a consortium of geosmin-degrading bacteria. *Water Research* 43:433–40. doi:10.1016/j.watres.2008.10.044.

Pahila, J. G., and E. E. S. Yap. 2013. Reduction of off-flavour compounds (geosmin and 2-methylisoborneol) using different organic acids. *Aquaculture, Aquarium, Conservation, and Legislation – International Journal of the Bioflux Society* 6:510–17. http://www.bioflux.com.ro/docs/2013.511-517.pdf.

Palsdottir, G., and P. C. Bishop. 1997. Nitrifying biotower upsets due to snails and their control. *Water Science and Technology* 36:247–54. doi:10.1016/S0273-1223(97)00331-4.

Pandey, P. K., B. Vivekanand, and K. Kumar. 2014. Biofilm in aquaculture production. *African Journal of Microbiology Research* 8 (13):1434–43. doi:10.5897/AJMR2013.6445.

Petersen, M. A., G. Hyldig, B. W. Strobel, N. H. Henriksen, and N. O. G. Jørgensen. 2011. Chemical and sensory quantification of geosmin and 2-methylisoborneol in rainbow trout (*Oncorhynchus mykiss*) from recirculated aquacultures in relation to concentrations in basin water. *Journal of Agricultural and Food Chemistry* 59 (23):12561–68. doi:10.1021/jf2033494.

Podduturi, R., M. A. Petersen, S. Mahmud, M. M. Rahman, and N. O. Jørgensen. 2017. Potential contribution of fish feed and phytoplankton to the content of volatile terpenes in cultured pangasius (*Pangasianodon hypophthalmus*) and tilapia (*Oreochromis niloticus*). *Journal of Agricultural and Food Chemistry* 65 (18):3730–36. doi:10.1021/acs.jafc.7b00497.

Rakocy, J. E., M. P. Masser, and T. M. Losordo. 2006. "Recirculating aquaculture tank production systems: Aquaponics – Integrating fish and plant culture." United States Department of Agriculture, Southern Regional Aquaculture Center Publication No. 454. Accessed April 25, 2018. https://srac-aquaponics.tamu.edu/serveFactSheet/10

Ramesh, M. R., K. M. Shanker, C. V. Mohan, and T. J. Varghese. 1999. Comparison of three plant substrates for enhancing carp growth through bacterial biofilm. *Aquacultural Engineering* 19:119–31. doi:10.1016/S0144-8609(98)00046-6.

Rintala, H., and A. Nevalainen. 2006. Quantitative measurement of streptomycetes using real-time PCR. *Journal of Environmental Monitoring* 8:745–49. doi:10.1039/b602485h.

Robertson, R. F., A. Hammond, K. Jauncey, M. C. M. Beveridge, and L. A. Lawton. 2006. An investigation into the occurrence of geosmin responsible for earthy-musty taints in UK

farmed rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 259:153–93. doi:10.1016/j.aquaculture.2004.11.046.

Robertson, R. F., K. Jauncey, M. C. M. Beveridge, and L. A. Lawton. 2005. Depuration rates and the sensory threshold concentration of geosmin responsible for earthy-musty taint in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 245:89–99. doi:10.1016/j.aquaculture.2004.11.045.

Robin, J., J.-P. Cravedi, A. Hillenweck, C. Deshayes, and D. Vallod. 2006. Off-flavor characterization and origin in French trout farming. *Aquaculture* 260:128–38. doi:10.1016/j.aquaculture.2006.05.058.

Schrader, K. K., S. Acuña-Rubio, R. H. Piedrahita, and A. M. Rimando. 2005. Geosmin and 2-methylisoborneol as the cause of earthy/musty off-flavors in cultured largemouth bass (*Micropterus salmoides*) and white sturgeon (*Acipenser transmontanus*). *North American Journal of Aquaculture* 67:177–80. doi:10.1577/A04-070.1.

Schrader, K. K., J. Davidson, and S. T. Summerfelt. 2013. Evaluation of the impact of nitrate-nitrogen levels in recirculating aquaculture systems on concentrations of the off-flavor compounds geosmin and 2-methylisoborneol in water and rainbow trout (*Oncorhynchus mykiss*). *Aquacultural Engineering* 57:126–30. doi:10.1016/j.aquaeng.2013.07.002.

Schrader, K. K., J. W. Davidson, A. M. Rimando, and S. T. Summerfelt. 2010. Evaluation of ozonation on levels of the off-flavor compounds geosmin and 2-methylisoborneol in water and rainbow trout *Oncorhynchus mykiss* from recirculating aquaculture systems. *Aquacultural Engineering* 43:46–50. doi:10.1016/j.aquaeng.2010.05.003.

Schrader, K. K., M. D. Harries, and P. N. Page. 2015. Temperature effects on biomass, geosmin, and 2-methylisoborneol production and cellular activity by *Nocardia* spp. and *Streptomyces* spp. isolated from rainbow trout recirculating aquaculture systems. *Journal of Industrial Microbiology Biotechnology* 42:759–67. doi:10.1007/s10295-015-1600-2.

Schrader, K. K., and S. T. Summerfelt. 2010. Distribution of off-flavor compounds and isolation of geosmin-producing bacteria in a series of water recirculating systems for rainbow trout culture. *North American Journal of Aquaculture* 72:1–9. doi:10.1577/A09-009.1.

Schram, E., J. Schrama, K. Kusters, C. Kwadijk, and A. Palstra. 2016. Effects of exercise and temperature on geosmin excretion by European eel (*Anguilla anguilla*). *Aquaculture* 451:390–95. doi:10.1016/j.aquaculture.2015.09.032.

Shrestha, M. K., and C. F. Knud-Hansen. 1994. Increasing attached microorganism biomass as a management strategy for Nile Tilapia (*Oreochromis niloticus*) production. *Aquacultural Engineering* 13:101–08. doi:10.1016/0144-8609(94)90007-8.

Spall, R. D., and R. C. Summerfelt. 1970. Life cycle of the white grub, *Posthodiplostomum mimimum* (MacCallum, 1921: Trematoda, Diplostomatidae), and observations on host-parasite relationships of the metacercaria in fish. In *A symposium on diseases and shellfishes. Special Publication No. 5*, ed. S. F. Sniesko, 218–30. Washington D.C.: American Fisheries Society.

Sugiura, N., and K. Nakano. 2000. Causative microorganisms for musty odor occurrence in the eutrophic Lake Kasumigaura. *Hydrobiologia* 434:145–50. doi:10.1023/A:1004000511610.

Summerfelt, S., J. Davidson, T. May, C. Good, and B. Vinci. March/April 2015. Emerging trends in salmonid RAS. Part II. System enhancements. *Global Aquaculture Advocate* 2015:64–65.

Terhune, J. S., D. J. Wise, J. L. Avery, L. H. Khoo, and A. E. Goodwin. 2003. "Infestations of the trematode *Bolbophorus* sp. in channel catfish." United States Department of Agriculture, Southern Regional Aquaculture Center Publication No. 1801. Accessed April 25, 2018. http://www.aces.edu/dept/fisheries/aquaculture/1801SRAC

Thompson, F. L., P. C. Abreu, and W. Wasielesky. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture* 203:263–78. doi:10.1016/S0044-8486(01)00642-1.

Tucker, C. S. 2000. Off-flavor problems in aquaculture. *Reviews in Fisheries Science* 8:45–88. doi:10.1080/10641260091129170.

Venable, D. L. 1998. "Control of the snail *Helisoma trivolvis*, an intermediate host of digenetic trematodes in catfish ponds." Master's thesis. Lafayette, LA. University of Southwestern Louisiana.

Wheaton, F. W., J. N. Hochheimer, and G. E. Kaiser. 1991. Principles of biological filtration. In *Engineering aspects of intensive aquaculture*, ed. M. B. Timmons, 1–31. Ithaca, NY: Northeast Regional Agricultural Engineering Service, Cooperative Extension.

Yamamoto, Y., T. Sakata, and K. Tanaka. 2000. Effect of light on geosmin production of myxobacterial strains isolated from eutrophic lakes. *International Association of Theoretical and Applied Limnology* 27:2132–34.